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Discocilia and Paddle Cilia in the Larvae of *Mulinia lateralis* and *Spisula solidissima* (Mollusca: Bivalvia)

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Abstract. The bivalve larval velum contains four bands of cilia: inner and outer preoral bands, an adoral band, and a postoral band. The preoral bands of compound cilia are generally considered to be used for both locomotion and food gathering. The adoral and postoral bands function in concert with the preoral bands in food gathering and transfer of food to the mouth. Cilia are usually described as cylindrical structures which taper to a blunt tip. Modified cilia with disc-shaped (discocilia) or paddle-shaped ends have been recorded in several invertebrate species. Here, for the first time, we demonstrate the presence of discocilia in the velum of *Mulinia lateralis* and paddle cilia in the velum of *Spisula solidissima*. Such cilia are restricted to the preoral bands and the central ciliary tuft. The presence of such cilia does not appear to increase the swimming velocity of these larvae in comparison to that of *Rangia cuneata* larvae of similar size. The possibility that these modified cilia have enhanced sensory capability remains to be tested.

Introduction

The larvae of bivalve molluscs are one of the major components of the meroplankton (Thorson, 1950). Most bivalve larvae develop from the fertilized egg to the veliger stage in twenty four hours or less. The veliger larva is characterized by a soft body enclosed by laterally compressed, semitransparent, paired valves and a protruding, oval, ciliated velum. The velum contains four bands of cilia: inner and outer preoral bands, an adoral band, and a postoral band (Elston, 1980; Waller, 1981). The preoral bands, consisting of compound cilia 20–80 μm long, are responsible for locomotion and food gathering. The adoral band, of shorter cilia approximately 8 μm long, transfer food particles to the mouth. The postoral band consists of complex cilia 15–20 μm in length. The efficiency of food concentration from the water depends on the harmonic beating of the preoral and postoral bands of cilia (Strathmann *et al.*, 1972; Strathmann and Leise, 1979).

Cilia are generally considered to be cylindrical structures with a constant diameter except for a tapering, blunt distal tip (Sleigh and Blake, 1977). Morphologically different cilia, discocilia, have been described for the polychaete *Lanice conchilega* (Pallas, 1776) by Heimler (1978). Tamarin *et al.* (1974) described cilia with "biconcave flattened discs or paddles," 1.33 μm in diameter in the ventral pedal groove of the juvenile mussel *Mytilus californianus* (Conrad, 1837) and ascribed to them a secretory function of adhesive material. Arnold and Williams-Arnold (1980) described discocilia in the embryo of the squid *Loligo pealei* (Lesueur, 1821); Matera and Davis (1982) observed paddle cilia in the rhinophore of the marine gastropod *Pleurobranchaea californica* (MacFarland, 1966); and ÓFoighil (1985) described papillae bearing cilia with bulbous tips of approximately 0.25 μm diameter in the mantle fold of temporary dwarf males of the bivalve *Pseudophythina rugifera* (Carpenter, 1864). In this report we describe, for the first time, the presence of modified cilia in the velum of larvae of two marine bivalves, the mactrids *Mulinia lateralis* (Say, 1882) and *Spisula solidissima* (Dillwyn, 1817).

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Materials and Methods

Mulinia lateralis adults were obtained from a cultured population at the Eastern Shore Laboratory of the Virginia Institute of Marine Science (VIMS), Wachapreague, VA. *Spisula solidissima* adults were obtained from a commercial fishing dock at Willis Wharf, VA. For comparison purposes a third mactrid, *Rangia cuneata* (Gray, 1831), was collected from the Rappahannock River, VA. All adult bivalves were maintained at the VIMS Wachapreague laboratory in water of appropriate salinity: 30 ppt for the marine stenohaline *S. solidissima*, 25 ppt for the euryhaline *M. lateralis*, and 10 ppt for the oligohaline *R. cuneata*. Adults were induced to spawn by thermal stimulation (24, 28, and 32°C for *S. solidissima*, *M. lateralis*, and *R. cuneata*, respectively). Larvae were cultured using the procedures of Culliney *et al.* (1975) and Chanley (1981) at the salinity of adult maintenance and temperatures of 23°C for *S. solidissima* and *M. lateralis*, and 25°C for *R. cuneata*. Water was changed every other day, at which time larvae were fed on mixtures of the phytoplankters *Pavlova* (*Monochrysis*) *lutheri* (Droop Green) (formerly *Monochrysis lutherii* Droop), *Isochrysis galbana* Parke, and *Isochrysis* aff. *galbana* (clone T-Iso). General procedures for phytoplankton culture followed the guidelines of Guillard (1983).

Preparation of larvae for scanning electron microscopy (SEM) followed the guidelines of Turner and Boyle (1975). Umbo stage larvae were siphoned from the culture container, retained on a 63 μm nylon mesh screen, thoroughly rinsed in 0.45 μm filtered water of the appropriate salinity, transferred to 10 ml of filtered seawater and relaxed by sequential additions of 1 ml of 8% (w/v) $MgCl_2$ in distilled water. Osmolarity of final relaxing solutions was not measured but relaxation was typically obtained following addition of 3–4 ml of $MgCl_2$ solution. Larvae were concentrated by centrifugation, and fixed for 2 hours with 2.5% chilled glutaraldehyde in distilled water buffered at pH 7.2 with 0.1 *M* sodium cacodylate. The fixative was subsequently pipetted off and the larvae subjected to three rinses, of 30 minutes each, of 3 ml of 0.1 *M* sodium cacodylate in 0.25 *M* NaCl. Larvae were post fixed for one hour in 5 ml of 1% OsO_4 in 0.19 *M* NaCl buffered at pH 7.2 with sodium cacodylate. Larvae were again rinsed, three times, in 0.1 *M* sodium cacodylate in 0.15 *M* NaCl, and stored overnight at 4°C. Dehydration was effected by 20-min exposures to a graded alcohol series (30, 50, 70, 90, 95, and 100%) followed by three changes in 100% acetone. Critical point drying was effected using a model 3000 Polaron dryer. Larvae were mounted on stubs using double sided adhesive tape, dessicated for a further 24 hours, coated with gold-palladium, and examined with an AMR model 1000 scanning electron microscope. Photographs were made with Polaroid 52 film.

Results

Figures 1 through 3 illustrate, with increasing magnification, the comparative morphology of the velum of the three species examined. In all species the postoral band consists of "typical" cylindrical cilia with distally tapering, blunted tips (Fig. 1A–C); however, the preoral bands exhibit species-specific differences. *Rangia cuneata* exhibit, again, "typical" cilia (Fig. 1C). The distal portions of the preoral cilia of *Spisula solidissima* and *Mulinia lateralis* terminate in biconcave paddles (Figs. 1A, 2A, 3A) or slightly inflated discs (discocilia, see Figs. 1B, 2B, 3B), respectively, the terminal structures measuring approximately 1–1.3 μm in diameter. A single preoral cilia in *M. lateralis* was observed with the disc 1–2 μm distal to the tip (Fig. 3B). The preoral cilia appear clustered and conform to the description of compound cilia as given by Waller (1981). The central ciliary tufts of the velum (Fig. 4) further exhibit species-specific cilia morphology; "typical" compound cilia in *R. cuneata* (Fig. 4B), cilia with terminal paddles in *S. solidissima*, and a mixture of cilia with terminal discs and discs 1–2 μm distal to the cilia tip in *M. lateralis* (Fig. 4A). In all three species the diameter of the ciliary shaft was between 0.2 and 0.4 μm.

Discussion

The comparatively rare occurrence of modified cilia in the animal kingdom prompts the question as to whether their presence is the result of artifacts during preparation for examination. Indeed, Ehlers and Ehlers (1978), examining flatworms, concluded that both paddle cilia and discocilia were absent in untreated tissue, but appeared only as artifacts after exposure to formaldehyde, sodium phosphate, and sodium cacodylate during preparation for SEM. Bergquist *et al.* (1977) contend that modified cilia observed in sponge larvae are real structures. Matera and Davis (1982), after a comprehensive study of *Pleurobranchaea californica* using light and transmission and scanning electron microscopy, rejected the conclusion of Ehlers and Ehlers (1978), noting that the paddle cilia can be seen with light microscopy in isosmotic seawater and made to straighten reversibly by exposure to hypertonic seawater. We also reject the conclusion of Ehlers and Ehlers (1978). In the present study discocilia or paddle cilia were a consistent characteristic of particular cilia bands within a species but not characteristic of the whole velum. Uniformity of cilia morphology throughout the velum, an observation consistent with the hypothesis of artifactural production during SEM preparation, was not

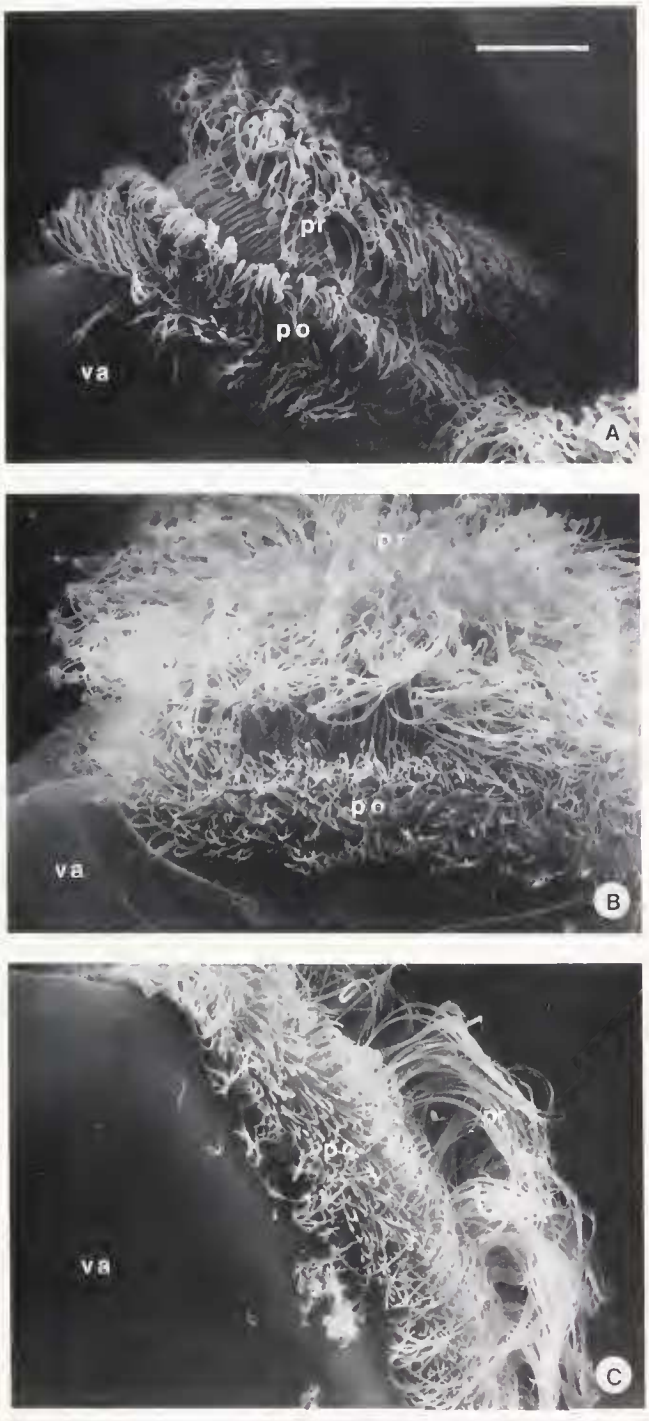

Figure 1. Scanning electron micrographs of the velum of (A) *Spisula solidissima*, (B) *Mulinia lateralis*, and (C) *Rangia cuneata* larvae. pr, preoral cilia; po, postoral cilia; va, valve. Scale bar = 20 μm

observed. The larvae used in this study were cultured at different salinities. The osmolarities of the final relaxing solutions were therefore different; however, larvae were fixed and subsequently processed identically and, in most instances, simultaneously. The question remains as to whether cilia morphology in these species will change with changing salinity; however, the ecological signifi-

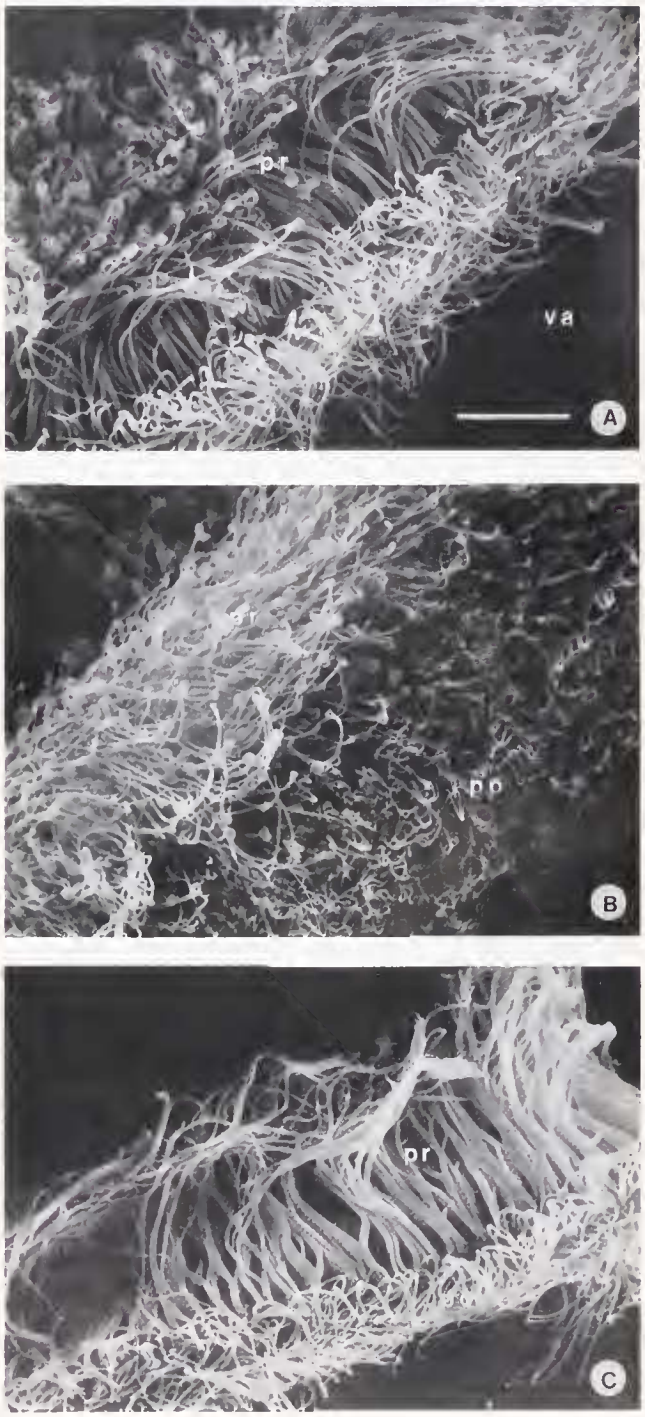

Figure 2. Scanning electron micrographs of the velum of (A) *Spisula solidissima*, (B) *Mulinia lateralis*, and (C) *Rangia cuneata* larvae. Details of the ciliary bands. pr, preoral cilia; po, postoral cilia; va, valve. Scale bar = 10 μm

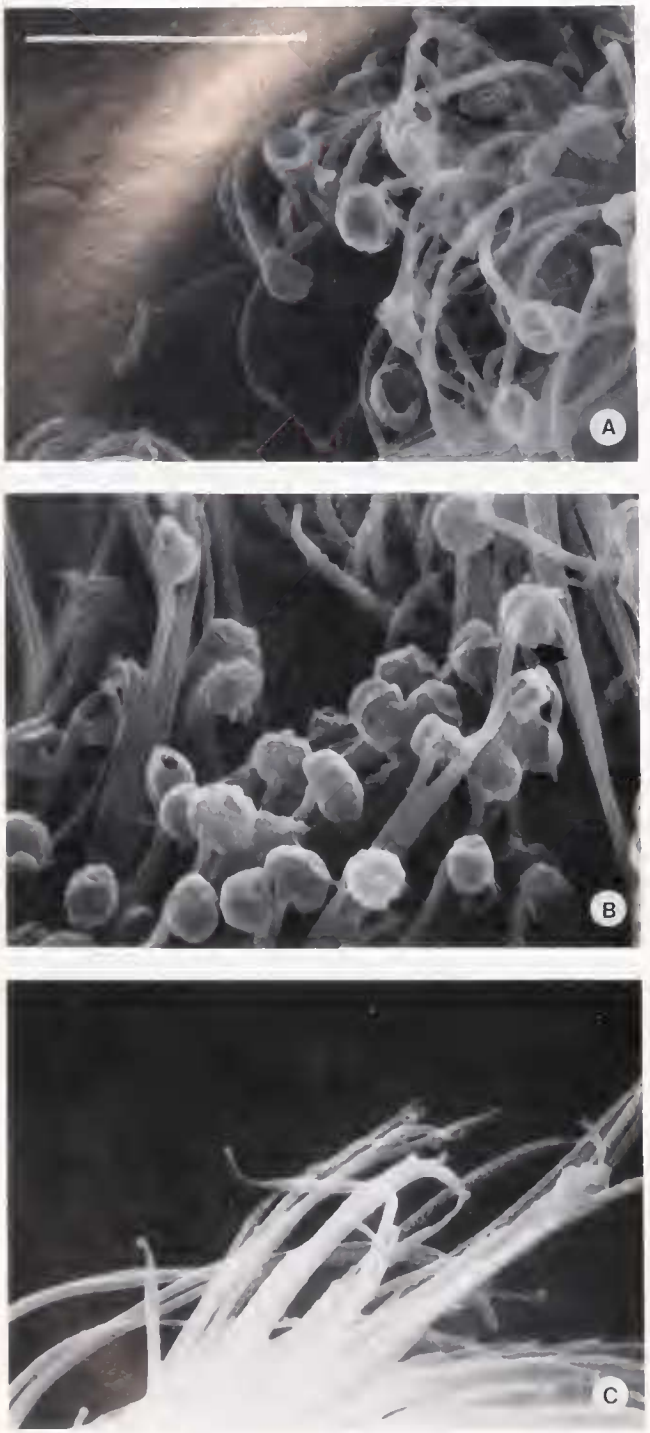

Figure 3. Scanning electron micrographs of the preoral velar cilia tips of (A) *Spisula solidissima*, (B) *Mulinia lateralis*, and (C) *Rangia cuneata* larvae. Note the biconcave paddles in *S. solidissima* and the terminal discs in *M. lateralis*. Arrow identifies a single disc distal to the cilia tip in *M. lateralis*. Scale bar = 10 μm

eance of this question is probably minimal in that both the larvae and adults of these species exhibit distinctly different salinity optima (Campos, 1988) which are reflected in the culture conditions used here. In summary, we believe the paddle and discocilia described here to be genuine structures.

The function of the modified velar cilia is debatable. Matera and Davis (1982) concluded that previous literature "collectively indicate that dilations of ciliary membranes represent a common morphological specialization subserving chemosensation." The structures described by ÓFoighil (1985) are also appropriately located for sensory function. However, they are slightly smaller than previously described cilia modifications. Only the "secretory" cilia described by Tamarin *et al.* (1974) are thought to have a primary function that is other than sensory. As mentioned earlier, the primary function of the preoral bands in the bivalve larval velum is generally considered to be in locomotion and food gathering. The sensory function has received little attention; however, consideration of veliger swimming behavior, wherein larvae progress in a vertically oriented helix with the velum extended in the direction of motion (see Cragg and Gruffydd, 1975; Cragg, 1980; Mann and Wolf, 1983), suggests that such a function is reasonable. The ability to combine locomotion and chemosensation to direct oriented movement along a gradient of chemostimulant has yet to be demonstrated in bivalve larvae. Chemosensory responses associated with settlement and metamorphic inducers have been demonstrated in well-mixed laboratory containers (*e.g.*, Coon *et al.*, 1985), but responses to gradients *per se* remain untested. Despite the apparent lack of an organized nervous system in velar tissue Elston (1980) suggests that "a cell to cell transmission of impulses" would fulfill this sensory function.

The presence of paddle or discocilia do not appear to enhance rate of movement in the species examined here. In larger animals, paddle structures would generally be considered advantageous in overcoming drag and enhancing propulsion. Pelagic bivalve veliger larvae generally range in size from 75 to 400 μm maximum dimension and move at absolute velocities of less than 10 mm s^{-1}. At this size and velocity, Reynolds numbers are less than or approach 1, a region where viscous forces predominate in determining maximal velocity (see Vogel, 1981). In a complementary study (Campos, 1988) a comparison of rates of vertical displacement (time to ascend through a unit vertical distance while swimming in a helical pattern) was made for three size ranges of larvae for each of the species examined here at the temperature and salinity of culture. The "D" or straight hinge veliger larvae of *R. cuneata*, *M. lateralis*, and *S. solidissima* exhibited mean (n = 25) rates of 0.38, 0.25, and 0.26 mm s^{-1}, respectively. Comparable values for umbone larvae were 0.49, 0.49, and 0.40 mm s^{-1}, respectively. Mean values for pediveliger larvae of the three species were 0.45, 0.34, and 0.40 mm s^{-1}, respectively. Despite the

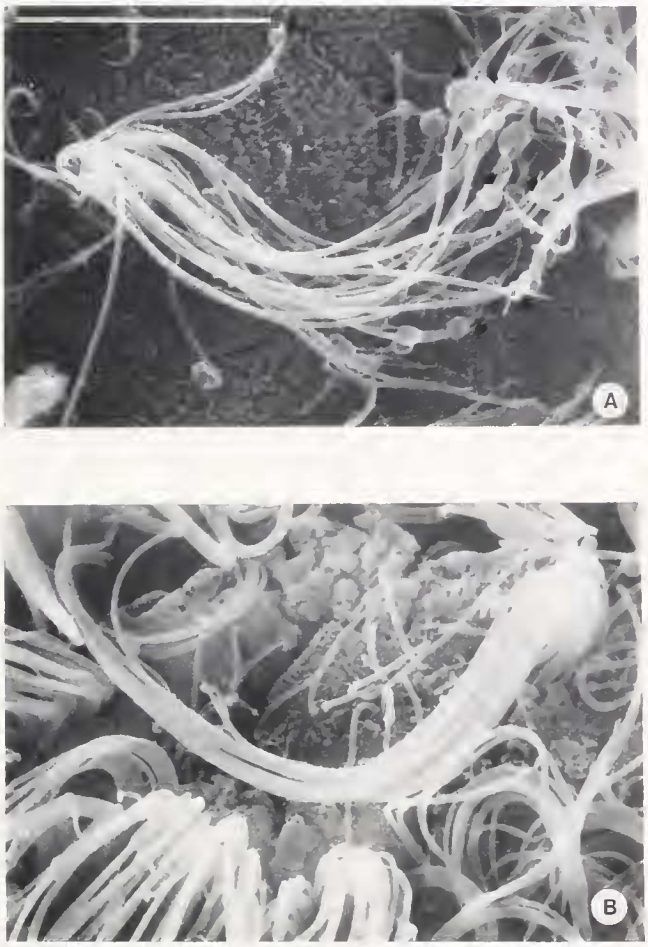

Figure 4. Scanning electron micrographs of the central ciliary tuft of (A) *Mulinia lateralis* and (B) *Rangia cuneata* larvae. Note the presence of both terminal discs and discs distal to the cilia tip (see arrows) in *M. lateralis*. Scale bar = 5 μm

fact that interspecific comparison is confounded by minor differences in morphometry, size, and, we suspect, specific gravity of larval stages, it is evident that the presence of discocilia and paddle cilia in *M. lateralis* and *S. solidissima*, respectively, does not apparently confer higher rates of vertical displacement when compared with *R. cuneata*. We did not compare absolute velocity (that which describes movement along the helical path rather than just vertical displacement) in the swimming study. Nonetheless, the ecologically meaningful value for vertical displacement (see discussion in Mann, 1986) suggests that the presence of modified cilia is not accompanied by greater ability to depth regulate in stratified water columns, an arguable advantage to any larvae encountering estuarine or shallow coastal environments.

Examination of previous descriptions of larvae or larval velar morphology for *Crassostrea virginica* Gmelin (Elston, 1980), *Ostrea edulis* Linne (Waller, 1981), and *Arctica islandica* Linne (Lutz *et al.*, 1982) have failed to demonstrate the presence of velar paddle cilia or discocilia—although in fairness only Waller (1981) provides micrographs of sufficient magnification and appropriate content for definitive statements. The taxonomic significance of these structures is also debatable. A significant component of bivalve taxonomy has historically focussed on adult shell characteristics and the present focus of larval taxonomy is on valve morphometry and hinge ultrastructure (see comments in Lutz *et al.*, 1982). Yet within one family, the Mactridae, we have examined three phylogenetically associated species and demonstrated the presence of three distinct cilia morphologies, each unique to one species. Clearly, determination of the frequency of occurrence, function, and taxonomic significance of these modified cilia in the bivalve larval velum awaits further investigation.

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